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Please send me copies of the following :

1. Chien, J. et al. Mol. and Cell. Endocrinology (2001) 181(1-2): 69-79
2. Chien, J. et al. Int. J. of Cancer (2001) 91(1): 46-54
3. Chien, J. et al. Oncogene (1999) 18(22): 3376-3382
4. Wong, E.C.C. et al. Proc. Amer. Assoc. for Cancer Res. (1997) 38: 288
5. Rayford, W. et al. Prostate (1997) 30(3): 160-166
6. Xue-Zhang, Q. et al. Endocrine (1995) 3(6): 445-451
7. Shah, G.V. et al. Endocrinology (1994) 134(2): 596-602
8. Rayford, W. et al. J. of Urology (1994) 151(5 suppl): 490A
9. Rayford, W.et al. J. of Urology (1993) 149(4 suppl): 479A
10. Shah, G.V. et al. Prostate (N.Y.) (1992) 21(2): 87-97
11. Sagol, O. et al. Annals of Medical Sciences (1999) 8(1): 14-21
12. Sussenot, O. et al. Prostate (1998) 36(suppl. 8): 43-51
13. Hanna, F.W. et al. J. Endocrinol. (1997) 152(2): 275-281
14. Sim, S.J. et al. Annals of Clinical and Laboratory Science (1996) 26(6): 487-495
15. Watanabe, K. et al. Fukushim J. Medical Science (1995) 41(2): 141-152
16. Esik, O. et al. European J. Gynaecological Oncology (1994) 15(3): 211-216

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Important: The number preceeding the title of a presentation is the abstract number

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PERINEAL COMPRESSION OF THE CORPUS SPONGIOSUM OF THE BULBAR URETHRA: AN OPERATION FOR POST RADICAL PROSTATECTOMY URINARY INCONTINENCE. Thomas A. Stamey, Stanford, CA (Presentation by Dr. Stamey)

Many excellent surgeons report a 5% rate of urinary incontinence after radical prostatectomy. Urinary incontinence is of greater concern to most patients than is erectile impotency since orgasmic function is always preserved. Artificial sphincters with circumferential compression of the bulbar urethra are often unsatisfactory because of infection, pressure erosion of the urethra and incomplete control of incontinence.

Based on our success with endoscopic suspension of the vesical neck in women with stress urinary incontinence, we have designed and tested a similar operation for men with post radical prostatectomy incontinence. Two broad bolsters of 6 mm diameter Dacron®, covered by a sleeve of Gore-Tex® to prevent stretching of the Dacron, are placed transversely across the bulbospongiosus muscle just distal to the superficial transversus perinei muscle. Both bolsters (3.5 cm long) are prepared prior to surgery with two long Prolene #1 sutures which anchor each end of each bolster. Four suprapubic needle passes (two on each side) of an extra long modified Stamey needle with two holes at the distal end (Greenwald Surg. Co., USA) are required to transfer the eight perineal sutures to the abdominal rectus fascia. Cystoscopy is required after each needle passage to insure absence of bladder or urethral perforation. Intraoperative perineal pressures of the spongy urethra must be about 100 cm of water for complete postoperative continence. A Stamey suprapubic tube (Cook Urol.) is left in place until all residual urine has dissipated, a process that can take as long as three months. All patients with their intraoperative and postoperative bulbar urethral pressures will be presented. The overall results are excellent although the follow-up remains short.

1050

MUSCARINIC RECEPTORS MAY ACT AS AGONIST-DEPENDENT ONCOGENES IN HUMAN PROSTATE CANCER. Walter Rayford, Girish V. Shah, and Mark J. Noble. KC, KS (Presented by Dr. Rayford)

Muscarinic receptors (MR) are primarily expressed in neurons and fully-differentiated cells. However, recent studies indicate these receptors can induce transformation when expressed in immature cells with proliferative capacity. MR are present in the human prostate and participate in the secretory function of the epithelium. Since the neuroendocrine (NE) cell population is significantly increased in prostate cancer (PC), it is likely that MR, in concert with other NE factors, may play a role in tumor progression. To test a possible role for MR in proliferation of PC, we studied the effects of carbachol on DNA synthesis in LnCaP cells. We also tested effects of carbachol on cytoplasmic Ca^{2+} transients.

Initially, effects of carbachol and other agents on the rate of 3H-thymidine incorporation or bromo deoxyuridine labeling were examined in cultured LnCaP cells. The cell proliferation rate was slowed by incubation in low-serum medium followed by a second in serum-free medium. Next, cells received various doses of carbachol ± atropine for 24 h. ^{3}H-thymidine was added 4 hours prior to termination, and incorporated ^{3}H-thymidine was quantified. In some experiments, LnCaP cells were preincubated with pertussis toxin (PTx) for 6 hours prior to addition of agonists. In a second group of experiments, effects of carbachol on cytoplasmic Ca^{2+} transients were examined. Cultured LnCaP cells were loaded with Indo-1 AM ester and ratio fluorescence measurements were made using 4 channel video fluorescence microscopy. The cells were excited by a xenon lamp and fluorescent images at 405 and 475 nm recorded on intensified CCD cameras after splitting the signal with dichroic mirrors. The 405 nm /475 nm fluorescence ratios were calculated as a function of time and $[Ca^{2+}]_i$ determined. Carbachol (0.1-10 nM) induced a dose-dependent increase in 3H-thymidine uptake. This was blocked by atropine implying the carbachol-induced increase was caused by activation of MR. PTx pre-treatment of LnCaP cells prevented this effect. Carbachol also induced a large increase in cytoplasmic Ca^{2+} transients of LnCaP cells. When considered together our results suggest carbachol-induced proliferation of LnCaP cells may be mediated through Gi-proteins and raise a possibility that Gi-mediated mechanisms may play an important role in proliferation of prostate cancer.

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ALTERED EXTRACELLULAR MATRICES DERIVED FROM BONE FIBROBLASTS INFLUENCE ANDROGEN RESPONSIVE GENES IN OVERLYING HUMAN PROSTATE CANCER CELLS. Michael H. Kane, Wei-Ping Shu, Jeffrey N. Gordon, Michael J. Droller, and Brian C.-S. Liu. New York, NY (Presentation to be made by Dr. Kane)

The prostatic epithelium, whether benign or malignant, resides in a complex environment. Recent evidence suggests that tissue specific alterations in gene expression may be related to cell-cell interactions and the influence of the underlying extracellular matrix (ECM). To investigate the hypothesis that ECM may regulate prostate cell behavior and androgen responsive genes, we have isolated and identified the ECM and its components from normal bone fibroblasts and from normal bone fibroblasts that were grown in the presence of 10 nM dihydrotestosterone (DHT).

Using Western blot analyses, we observed that the DHT treated bone fibroblasts expressed greater type IV collagen than the untreated bone fibroblasts. Furthermore, DHT treated bone fibroblasts have a decrease in laminin and fibronectin when compared with the untreated bone fibroblasts.

Human prostatic cancer LNCaP cells were grown on ECM derived from untreated and DHT treated bone fibroblast cells in the absence of exogenous DHT, and the expression of prostate specific antigen (PSA) was determined. We observed that PSA was up-regulated (more than 5-fold) when the LNCaP cells were grown on the ECM derived from DHT treated bone fibroblasts even in the absence of exogenous DHT. The expression of PSA was not up-regulated when the LNCaP cells were grown on ECM derived from untreated bone fibroblasts. Furthermore, when the LNCaP cells were grown on TransWell filters, which separated the cells from the ECM derived from DHT treated bone fibroblasts, no increase in PSA expression was detected.

To determine the mechanism in which the ECM may regulate PSA expression in the LNCaP cells, the expression of androgen receptors on the LNCaP cells was determined. Using reverse transcription polymerase chain reaction (RT-PCR) and Western blot analyses, we showed that when the LNCaP cells were grown on plastic culture dishes in the presence of 10 nM DHT, an increase in androgen receptor protein was observed. This was followed by a down-regulation of the androgen receptor message. When the LNCaP cells were grown on ECM of untreated bone fibroblasts, no detectable increase in androgen receptor proteins was observed, and the androgen receptor message (mRNA) was at the same level of expression as LNCaP cells grown on plastic culture dishes without the presence of DHT. However, when the LNCaP cells were grown on ECM derived from DHT treated bone fibroblasts, an up-regulation of androgen receptor proteins was demonstrated on Western blot, and the androgen receptor mRNA was shown to be down-regulated when assayed by RT-PCR.

When the LNCaP cells were grown on TransWell filters, which separated the cells from the ECM derived from DHT treated bone fibroblasts, no increase in androgen receptor proteins was observed. Furthermore, no down-regulation of androgen receptor mRNA was detected when the LNCaP cells were grown on TransWell filters.

The above results suggest that DHT has both a direct and an indirect effect on LNCaP cells, and may act via the extracellular matrix components. These results may also partially explain the clinical observation that bone provides a fertile soil for prostate cancer growth, proliferation, and metastasis.

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MORPHOLOGICAL AND FUNCTIONAL CYTODIFFERENTIATION OF THE DUNNING PROSTATIC ADENOCARCINOMA.
Nobs Hayashi*, Yoshiki Sugimura, Juichi Kawamura and Gerald R. Cunha* Tsu, Mie, Japan and San Francisco, CA. (Presentation by Dr Hayashi)

Mesenchyme plays a critical role in inducing epithelial morphogenesis and cytodifferentiation during normal prostatic development. Likewise, mesenchyme can induce completely new morphological and functional expression in normal adult epithelial cells. The responsiveness of normal adult epithelial cells to mesenchymal inductors has led to the observations that seminal vesicle mesenchyme (SVM) can induce the Dunning prostatic adenocarcinoma epithelial cells (DT-E) to differentiate with a concomitant reduction in tumorigenesis.

Previous SVM+DT-E experiments utilized small 0.5 mm DT fragments. In the present experiments DT-E was purified from DT cell suspensions by Percoll gradient centrifugation and recombined with rat neonatal SVM. The resultant tissue recombinants (SVM+DT-E) were grafted under renal capsules of male athymic mice and grown for 2 months.

Under these conditions SVM induced the DT-E to exhibit a highly differentiated secretory phenotype by forming ducts lined with tall columnar epithelial cells or large clear cells with pale cytoplasm. Whereas control grafts of the DT by itself formed large tumors (> 1000 mm^3) during the 2 month growth period, the SVM+DT-E recombinants survived but remained small (< 30 mm^3). The loss of tumorigenicity in SVM+DT-E recombinants was associated with a striking reduction of epithelial ^{3}H-thymidine labelling index in SVM+DT-E recombinants (DT : 6.31%, SVM+DT-E recombinants : 0.80%). Differences in secretory proteins were also observed in SVM+DT-E recombinants in comparison to DT. Examination of testosterone metabolism in grafts of DT versus SVM+DT-E recombinants by thin layer chromatography with [1β, 2β-^{3}H] testosterone revealed that the major metabolite in DT-E was Δ^4-Adione, otherwise that of epithelium from SVM+DT-E recombinants was DHT similar to dorsal prostate and seminal vesicle epithelium.

The above SVM-induced changes in DT-E suggest the possibility that emerging or established carcinomas might be regulated at least in part by their connective tissue microenvironment.